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RESEARCH NOTE

Preceding infections and anti-ganglioside antibodies in patients with Guillain–Barré syndrome: a single centre prospective case-control study

S. Sinha[1], K. N. Prasad[1], D. Jain[1], C. M. Pandey[2], S. Jha[3] and S. Pradhan[3]

Departments of [1]Microbiology, [2]Biostatistics and [3]Neurology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

ABSTRACT

Preceding infections and anti-ganglioside antibodies were assessed among 80 Guillain–Barré syndrome (GBS) patients and 125 controls. Previous infections were more frequent among GBS patients than among controls ($p < 0.0001$), and had a significant association with axonal subtype compared with acute inflammatory demyelinating polyneuropathy (AIDP) (29/46 vs. 10/34 patients; $p < 0.05$). *Campylobacter jejuni* (26%) was the most common preceding infection among GBS patients, followed by *Mycoplasma pneumoniae* (15%). Anti-ganglioside antibodies were detected more frequently among GBS patients than among controls (65/80 vs. 13/125; $p < 0.001$), and a higher proportion of axonal cases had these antibodies than did AIDP patients (43/46 vs. 22/34; $p < 0.01$).

Keywords Anti-ganglioside antibodies, axonal subtype, *Campylobacter jejuni*, Guillain–Barré syndrome, preceding infections

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Corresponding author and reprint requests: K. N. Prasad, Department of Microbiology, SGPGIMS, Lucknow, India
E-mail: knprasad@sgpgi.ac.in

Guillain–Barré syndrome (GBS) is a common cause of acute flaccid paralysis, with an annual incidence of 1–2 cases/100 000 population [1]. The pathological spectrum of GBS includes acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN) and acute motor sensory axonal neuropathy (AMSAN). One-half to two-thirds of GBS patients usually report antecedent infections, and *Campylobacter jejuni*, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and *Mycoplasma pneumoniae* are recognised triggering agents of GBS [2]. Anti-ganglioside antibodies are believed to play an important role in the pathogenesis of GBS because of molecular mimicry between some glycoconjugate epitopes of microbes and the nerve tissue of the host [3]. The present study investigated commonly described microbial infections and anti-ganglioside antibodies in patients grouped according to GBS subtype.

Eighty GBS patients admitted to the neurology ward, 80 healthy volunteers matched for age and gender, and without any history of apparent infectious illness at the time of sample collection, and 45 patients with neurological diseases other than GBS, were included in a prospective case-control study between February 2001 and March 2005. GBS patients were defined according to criteria described previously [4] and were further subdivided into GBS subtypes [5]. Single stool and serum samples were collected from all GBS patients and controls with other neurological diseases within 24–48 h of admission.

Previous *C. jejuni*, *M. pneumoniae*, CMV, EBV and herpes simplex virus (HSV) infections were assessed in all GBS patients and controls. *C. jejuni* infection was detected by stool culture and PCR [6] and by serum antibodies [2]. Serum dilutions used were 1:500 and 1:1000 for IgM and IgG, respectively. *C. jejuni* infection was defined by at least one of the following criteria: (i) stool culture and/or PCR positive [6]; (ii) presence of IgG and IgM antibodies; and (iii) high titre IgG antibody with a history of diarrhoea within the 4-week period preceding the onset of GBS [7]. Other microbial infections were detected using commercially available IgM ELISA kits. CMV infection was confirmed by measuring IgG avidity. Anti-ganglioside antibodies against GM1, GD1a, GD1b, GT1b, GM2 and GalC in the sera of patients and controls were detected by ELISA according to standard protocols [8,9].

Comparisons between the groups were made using *Z*-tests for proportions and independent two-tailed *t*-tests, with $p < 0.05$ considered to be significant. Multiple logistic regression was used to assess independent factors associated with GBS. SPSS v.13.0 software (SPSS Inc., Chicago, IL, USA) was used for data management and analysis.

Demographical details of GBS patients and controls are summarised in Table 1. The axonal form (AMAN and AMSAN) of GBS was more common among younger patients than AIDP (mean age, axonal vs. AIDP, 25.5 ± 17.4 vs. 37.2 ± 21.6 years; $p < 0.001$). Thirty-eight (48%) GBS patients had a clinical history of at least one recent infection (Table 1). AIDP and axonal subtypes were more common among patients with previous respiratory infection ($p < 0.05$) and diarrhoea ($p < 0.05$). Evidence of preceding infection was documented in 39 (49%) GBS patients and ten (8%) controls (Table 1), with *C. jejuni* (26%) and *M. pneumoniae* (15%) infections being associated strongly with GBS (Table 1). *C. jejuni* infection was more frequent in association with the axonal subtype than with the demyelinating subtype ($p < 0.01$). CMV, EBV and HSV infections were present in 6%, 4% and 1% of GBS patients, respectively.

One or more classes of anti-ganglioside antibodies were present among 65 (81%) GBS patients and 13 (10%) controls ($p < 0.001$). More GBS patients with the axonal subtype had anti-ganglioside antibodies than did AIDP patients (43/46, 93%, vs. 22/34, 66%; $p < 0.01$). Anti-GM1 and anti-GD1a IgG antibodies were associated with the axonal subtype (Table 2). In multiple logistic regression, antecedent *C. jejuni* infection (OR 5.9), anti-GM1 IgM (OR 9.6), anti-GM1 IgG (OR 11.9), anti-GD1a IgG (OR 9.6) and anti-AsGM1 IgG (OR 5.1) were associated independently with GBS.

Table 1. Demographical data and spectrum of infections for patients with Guillain–Barré syndrome (GBS) and controls

	Subjects					
	GBS (*n* = 80)					
		Axonal (*n* = 46)				
Demographical parameters	**AIDP**	**AMAN**	**AMSAN**	**Total**	**HC**	**DC**
Number of subjects	34 (43%)	27 (33%)	19 (24%)	46 (57%)	80	45
Mean age (years)	37.2 ± 21.6	26.3 ± 17.5	25.0 ± 17.4	25.5 ± 17.4	32.4 ± 7.26	34.3 ± 16.9
Gender (M:F)	26:8	20:7	11:8	31:15	58:22	34:11
Preceding illness						
Respiratory infection (*n* = 17)[a]	12 (35%)	1 (4%)	4 (21%)	5 (11%)	0	2 (4%)
Diarrhoea with or without abdominal pain (*n* = 10)[b]	1 (3%)	6 (22%)	3 (16%)	9 (20%)	0	0
Fever (*n* = 11)	4 (12%)	5 (19%)	2 (11%)	7 (15%)	0	0
Chicken pox (*n* = 1)	1 (1%)	0	0	0	0	0
Infections						
Campylobacter jejuni[c]	2 (6%)	13 (48%)	6 (32%)	19 (41%)	4 (5%)	2 (4%)
Mycoplasma pneumoniae[d]	7 (21%)	3 (11%)	2 (11%)	5 (11%)	1 (1%)	1 (2%)
CMV	1 (3%)	3 (11%)	1 (5%)	4 (9%)	1 (1%)	1 (2%)
EBV	0	2 (7%)	1 (5%)	3 (7%)	0	0
HSV	0	0	1 (5%)	1 (2%)	0	0

HC, healthy controls; DC, controls with other neurological diseases.
AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor sensory axonal neuropathy; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus.
[a]Respiratory infection, AIDP vs. axonal, $p < 0.05$.
[b]Diarrhoea, axonal vs. AIDP, $p < 0.05$;
[c]*C. jejuni* infection, GBS vs. DC, $p < 0.001$; GBS vs. HC, $p < 0.001$; axonal vs.AIDP, $p < 0.01$.
[d]*M. pneumoniae* infection, GBS vs. DC, $p < 0.05$; GBS vs. HC, $p < 0.01$.

Table 2. Frequency of IgM and IgG anti-ganglioside antibodies in patients with Guillain–Barré syndrome (GBS) and controls

	GBS			Controls		
Anti-ganglioside antibody	**Axonal[a] (*n* = 46)**	**AIDP (*n* = 34)**	**Total (*n* = 80)**	**DC (*n* = 45)**	**HC (*n* = 80)**	**Total (*n* = 125)**
Any class	43[b] (93%)	22 [b] (65%)	65 (81%)[c]	10 (22%)	3 (4%)	13 (10%)[c]
Both classes	24 (52%)	9 (26%)	33 (41%)	3 (7%)	0	3 (2%)
Only IgM	2 (4%)	2 (6%)	4 (5%)	1 (2%)	0	1 (1%)
Only IgG	17 (40%)	11 (32%)	28 (35%)	6 (13%)	3 (4%)	9 (7%)
IgG						
Anti- GM1[d]	30 (65%)	11 (32%)	41 (51%)	3 (7%)	3 (4%)	6 (5%)
Anti-GD1a[e]	24 (52%)	4 (12%)	28 (35%)	2 (4%)	0	2 (2%)
Anti-GD1b	7 (15%)	5 (15%)	12 (15%)	0	0	0
Anti-GT1b	8 (7%)	2 (21%)	10 (13%)	0	0	0
Anti-GM2	4 (9%)	3 (9%)	7 (9%)	4 (9%)	0	4 (3%)
Anti-GalC	5 (11%)	6 (18%)	11 (14%)	0	0	0
Anti-AsGM1	11 (24%)	5 (15%)	16 (20%)	0	0	0
IgM						
Anti-GM1	21 (46%)	10 (29%)	31 (39%)	2 (4%)	0	2 (2%)
Anti-GD1a	14 (31%)	1 (3%)	15 (19%)	1 (2%)	0	1 (1%)
Anti-GD1b	1 (2%)	0	1 (1%)	0	0	0
Anti-GT1b	2 (4%)	0	2 (3%)	0	0	0
Anti-GM2	4 (9%)	3 (9%)	7 (9%)	1 (2%)	0	1 (1%)
Anti-GalC	1 (2%)	2 (6%)	3 (4%)	0	0	0
Anti-AsGM1	1 (2%)	1 (3%)	2 (3%)	0	0	0

HC, healthy controls; DC, controls with other neurological diseases; AIDP, acute inflammatory demyelinating polyneuropathy.
[a]Axonal includes acute motor axonal neuropathy (AMAN) and acute motor sensory axonal neuropathy (AMSAN).
[b]Axonal vs. AIDP, $p < 0.01$.
[c]GBS vs. DC, $p < 0.001$; GBS vs. HC, $p < 0.001$.
[d]GM1 anti-ganglioside IgG antibodies, axonal vs. AIDP $p < 0.01$.
[e]GD1a anti-ganglioside IgG antibodies, axonal vs. AIDP $p < 0.01$.

Both axonal and AIDP subtypes were present equally in the population investigated, which is in contrast to studies from western and east/Asian countries [10]. GBS was more common among males than among females, but was not associated with age; however, the axonal subtype affected younger populations significantly more frequently ($p < 0.001$), which is a finding not reported previously.

Antecedent respiratory illness and diarrhoea were associated significantly with AIDP and axonal subtype, respectively. Although recent infections have been reported in 50–70% of GBS patients [11], associations between clinical symptoms and GBS subtypes are not well-documented. It is also believed that sub-clinical infections could also trigger GBS [6]. Microbiological evidence of infection was detected in significantly higher numbers of GBS patients (49%) than in both groups of controls (8%). *C. jejuni* and *M. pneumoniae* infections were associated with GBS, with GBS patients having a high *M. pneumoniae* infection rate (15%) compared with other published series (5–6%) [12]. *C. jejuni* infection was present in 26% of GBS patients, similar to the figures of 13–39% reported from Europe and north America, but much lower than those reported from China and Japan [2]. As in other east/Asian countries [13,14], *C. jejuni* infection was linked strongly to axonal subtype. Although CMV (6%) and EBV (2%) infection rates in the present study were within the ranges reported previously [2,12,15], the proportions of both differed from those of the controls. In contrast to a previous study [12], four of five CMV-positive GBS patients had axonal degeneration with no cranial nerve involvement. HSV infection did not appear to be common in the GBS patients studied.

Anti-ganglioside antibodies were found significantly more often in GBS patients than in controls, and more patients with the axonal subtype had anti-ganglioside antibodies than did patients with the demyelinating subtype (Table 2). Anti-GM1 IgG and IgM, and anti-GD1a IgG antibodies, were detected more frequently in GBS patients. Anti-GM1 IgG antibodies have been found in >50% of GBS patients, irrespective of the infectious serology [14], with a prevalence of 0–81% [16]. Anti-GM1 IgG and anti-GD1a IgG antibodies were associated significantly with the axonal subtype. It has been reported that anti-GM1 IgG and anti-GD1a IgG are relatively specific markers for axonal GBS [17], but several studies have failed to correlate anti-GM1 antibodies with electrodiagnostic findings [8,13,18]. It has been speculated that anti-GD1a IgG may have a more important role in the pathogenesis of axonal GBS [19].

In conclusion, the axonal subtype of GBS affects the younger population more commonly. In addition to *C. jejuni*, *M. pneumoniae* infection appears to be associated strongly with GBS cases. Anti-ganglioside antibodies are detected more frequently in cases of GBS, especially with the axonal subtype.

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